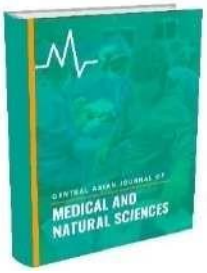
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES




Volume: 04 Issue: 06 | Nov-Dec 2023 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

# The Relationship of Contraceptive Pills and Breast Cancer as a Histopathological Study in Al-Muthanna Governorate – Iraq

1. **Kholoud Ayed Alzayadi**
2. **Bassem Abdullah Jassim**
3. **Hanaa Ali Aziz**

*Received 15th Oct 2023,*
*Accepted 10thNov 2023,*
*Online 15th Nov 2023*

[1,3]Collage of Science, Al-Muthanna University, Iraq

[2]Collage of Veterinary Medicine, Al-Muthanna University, Iraq

**Key words:** Breast cancer, contraceptive, Histopathological, IHC, Progesterone, Ki67.

**Abstract:** Cancer is a highly lethal illness that impacts communities globally, and among women worldwide, breast cancer ranks as the most frequently diagnosed form of cancer. In Iraq, breast cancer stands as the second leading cause of mortality, following closely behind cardiovascular diseases. The aim of this current study was to investigate the histological and morphological alterations occurring in the breast tissue of individuals diagnosed with breast cancer. In this study, a total of (60 clinical samples) were included, encompassing both older and younger women diagnosed with breast cancer, who were patients at Al-Sadiq Hospital and daily clinics in Al-Muthanaa province. The research was conducted over the period from November 2022 to May 2023. The sampling phase spanned from November 2022 to May 2023. Total of sixty samples were gathered and categorized into three distinct groups: a breast cancer patients' group 30-39 years old, a patients at 40-49' group ,and a third group 50-60 years old. The age range for both the patient and healthy groups spanned from (30 to 60 )years. In the findings of our study, we observed a slight increase in the risk of breast cancer in women who use contraceptives compared to those who abstain from using them, we detected notable histological and morphological alterations in the breast tissue when contrasted with healthy breast tissue. These changes encompassed abnormal cell growth, enlargement of breast structures, the presence of anomalous cell clusters, congested blood vessels, elongated regions with sporadic bleeding, necrotic lesions, and various other conspicuous modifications, all indicative of the existence of breast cancer. The results of the immunohistochemical analysis indicated a notable disparity in cell proliferation between the group of healthy subjects and the group of infected individuals.

## Introduction

Cancer poses a major global health issue and stands as the second leading cause of death worldwide. It comprises a group of disorders marked by the uncontrollable multiplication and dissemination of irregular cells. When not addressed, this unregulated expansion can result in lethal consequences [1]. Breast cancer is the leading cancer type affecting women and is regarded as the second most frequent cause of mortality. Over time, there has been a noticeable increase in the worldwide occurrence of breast cancer, with an annual tally of more than one million new instances reported [2]. Breast cancer encompasses a range of conditions in which the cells within breast tissue undergo uncontrolled proliferation, leading to the development of an anomalous mass or tumor [3,4]. While numerous women have one or more risk factors associated with breast cancer, most of them never actually develop the disease. Conversely, many women diagnosed with breast cancer do not have any identifiable risk factors. Some risk factors, like age or ethnicity, cannot be altered, while others are linked to environmental factors that may lead to cancer or individual behaviors. The critical factors contributing to breast cancer encompass gender, age, ethnicity, family history, genetic predisposition, breast density, obesity, menstrual patterns, pregnancy, breastfeeding, exposure to radiation, use of ovulation-stimulating medications, as well as alcohol and tobacco use [5,6].

The contraceptive as hormonal drugs that supporting last findings of an approximately 20% which increased the breast cancer dangerous for women that used the contraceptive pills or that recent use the hormonal contraceptive drugs. Recent research also determined this risks which increasing with duration of used of hormonal drugs as contraceptive pills, the risks may continue for more than five years in women that take the contraceptive pills[7].

Histopathologically, the primary characteristic is the abundant presence of numerous giant cells within the stromal tissue, and occasionally within the glandular lumina. It's important to note that these giant cells are not considered neoplastic . These giant cells are notably large, possessing ample cytoplasm and containing multiple centrally located nuclei, often with visible nucleoli. The carcinoma cells may be organized in various structures like tubules, glands, papillae, and may also exhibit mucinous or lobular arrangements. The stromal tissue contains several inflammatory cells, including lymphocytes, monocytes, and histiocytes, in addition to numerous extra-vascular red blood cells and hemosiderin deposits. These hemosiderin deposits are definitively responsible for the distinctive brownish coloration of the tumor [8].

Immunohistochemistry is a method employed to identify cellular or tissue components (antigens) by observing the interaction between antigens and antibodies. This interaction and the specific binding sites of antibodies can be detected either by directly labeling the antibodies or by utilizing a secondary labeling technique [9].

## Materials and Method

### Methods:

This research involved a total of (60) clinical samples, comprising both older and younger women with breast cancer who were patients at private Al-Sadiq Hospital and daily clinics in Al-Muthanaa province. The study was conducted from November 2022 to May 2023, and The sampling phase spanned from November 2022 to May 2023. Total of sixty samples were gathered and categorized into three distinct groups: a breast cancer patients' group 30-39 years old a patients at 40-49' group, and a third group 50-60 years old. The age range for both the patients and the healthy individuals ranged from (30 to 60) years. After conducting a comprehensive examination of the health records for each sample and confirming the histological diagnosis as malignant, we removed any cases that did not meet the study criteria or were not related to the disease. This procedure was carried out after obtaining official approvals from the hospital administration and the attending physician and obtaining the comprehensive and informed consent of the patients, with a commitment to protecting patient confidentiality.

**Biopsy Samples:**

The biopsy was placed in a plastic tube containing 10% neutral buffered formalin (NBF) before being processed via an automated tissue processor (Histoline, Italy) in Dr. Haider Khudair's laboratory in Muthanna, Iraq. This was done to generate formalin-fixed, paraffin-embedded (FFPE) blocks for subsequent histological examinations. After removing the organs from the patients, the tissue samples were swiftly collected and immediately immersed in 10% formalin for fixation. Following the fixation process, the samples were carefully trimmed using a specialized scalpel to prepare them for placement in labeled tissue cassettes. To eliminate the fixation agent and water from the tissues, the samples underwent a series of alcohol baths with varying concentrations (70%, 95%, and 100%). Subsequently, the tissue-containing paraffin block was precision-trimmed to expose the specific area of interest and then sliced into fine sections measuring approximately 5 micrometers in diameter using a microtome machine, to enhance the visibility and clarity of the tissue structures for assessment, both hematoxylin and eosin histochemical stains (H&E) were typically applied to the tissue sections. For the immunohistochemistry (IHC) analysis, thin sections measuring 3-4 micrometers were cut from the paraffin blocks containing the specimens. These sections were then stained using the BenchMark ULTRA immunohistostainer, employing pragesterone and ki67 kits for the study.

**Statistical Analysis**

A statistical analysis was carried out to examine differences among different groups using (SPSS version 25). The mean of the data was assessed using ANOVA, and statistically significant distinctions were identified at a significance level of ($P \leq 0.05$).

**Results :**

**Demographic characteristics:**

In this study, as shown in Figure 1, it was evident that there were no statistically significant variations, with a significance level of $P<0.05$, in the average ages when comparing the patient groups to the control group ($P = 0.941$). Similarly, no significant differences at $P<0.05$ were observed across all age groups when comparing patients to the control groups ($P = 0.739$). Additionally, there was no significant disparity at $P<0.05$ in terms of residence (urban and rural) between the patient and control groups ($P=0.654$). It's crucial to emphasize that in this case-control study, in order to mitigate any potential biases associated with age and residency, it is essential to confirm that there are no statistically significant differences in the distribution of individuals between the two groups concerning age and residency.

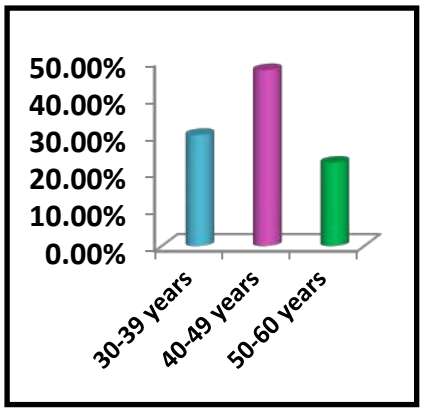



**Figure (1): Distribution of patients with Breast Cancer according to age groups**

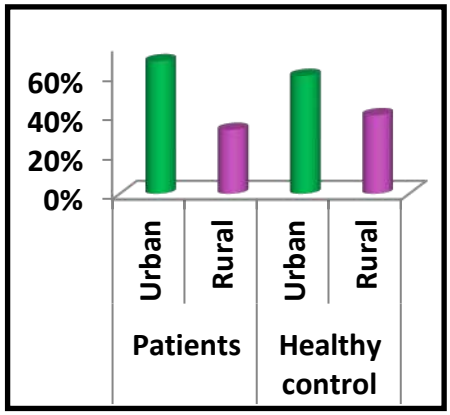



**Figure (2): Distribution of patients with control according to residence**

The current findings indicate that of individuals diagnosed with breast cancer there were 20(50.0%) individuals who used contraceptives, and 20(50.0%) individuals who did not use contraceptives, as illustrated in Table (1). However, it's worth noting that the difference between the two groups was not found to be statistically significant (p=0.464).

**Table (1): Frequency distribution patients with breast cancer and healthy control according . to contraceptive**

| Characteristic | Patients n = 40 | Healthy control n=20 | P |
|---|---|---|---|
| **Contraceptive** | | | |
| **Yes, n (%)** | 20 (50.0 %) | 12 (60.0 %) | 0.464 ¥ NS |
| **No, n (%)** | 20 (50.0%) | 8 (40.0%) | |

n: number of cases; Chi-square test; NS: not significant at P > 0.05

The current findings indicate that (22.5%) of individuals diagnosed with breast cancer have a positive family history of the disease. In contrast, (77.5%) of patients do not have a family history of breast cancer, as illustrated in Figure (3).

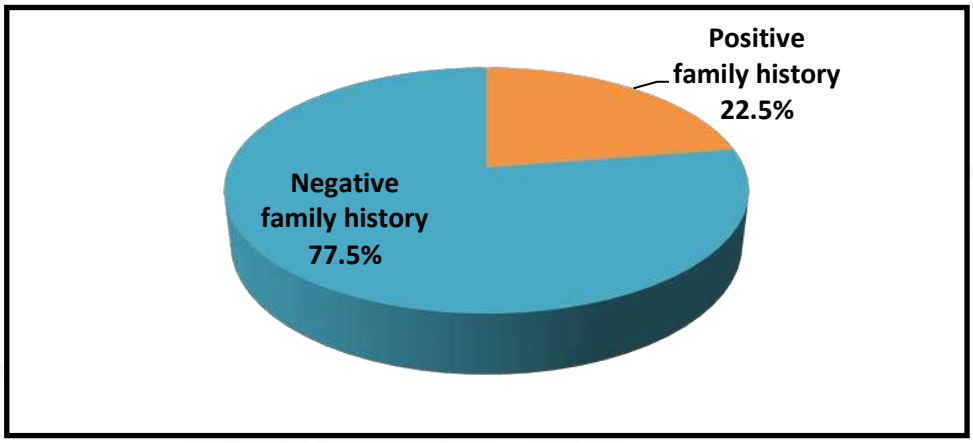
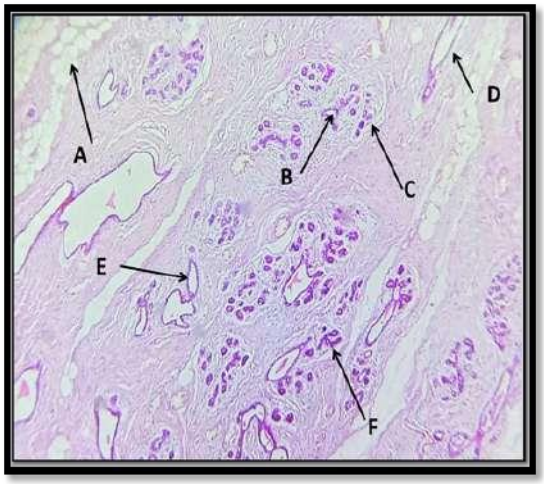



**Figure (3): Distribution of patients according to family history.**

**Histological Examination:**

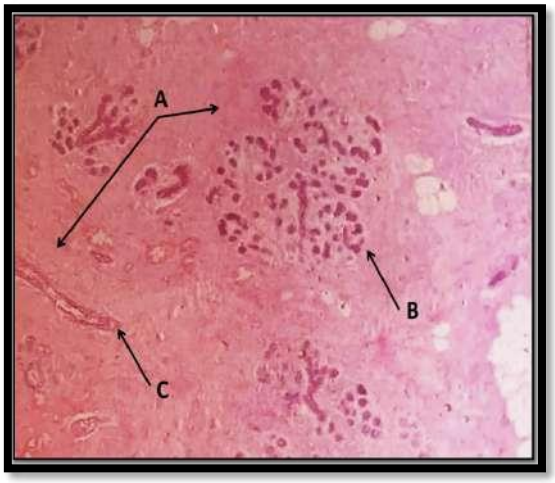
The histological examination of breast tissue from women who had an infection revealed significant changes. These changes included abnormal cellular growth occurring in different areas within the breast parenchyma. Additionally, there was an increased presence of cellular material between the tissue fibers that typically separate the lobules. Furthermore, the tissue exhibited numerous irregular spaces, some filled with thick secretions and others containing blood, These alterations vary in comparison to the control group Figure(4&5). These findings are illustrated in Figure (6)




**Figure(4): Tissue section of normal breast shows:**

**A- adipose tissue, B- interlobular ducts, C- lobule, D- lactiferous ducts, E- terminal interlobular ducts, F-acini**

**H&E (4X )**




**Figure(5) :Tissue section of normal breast shows:**

**A- connective tissue (stroma), B- acini, C-lactiferous ducts H&E (4X).**

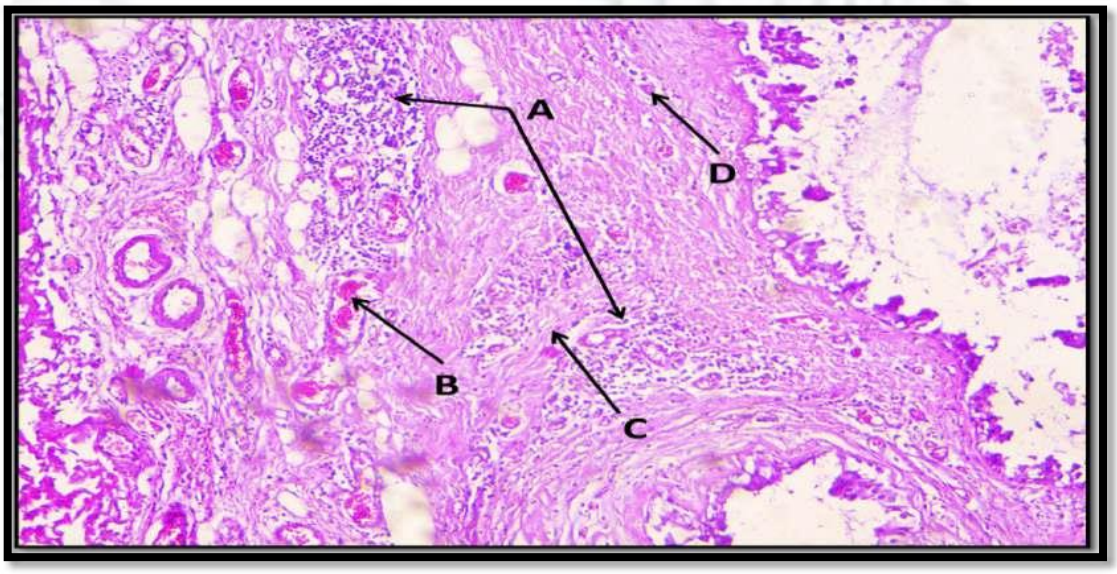
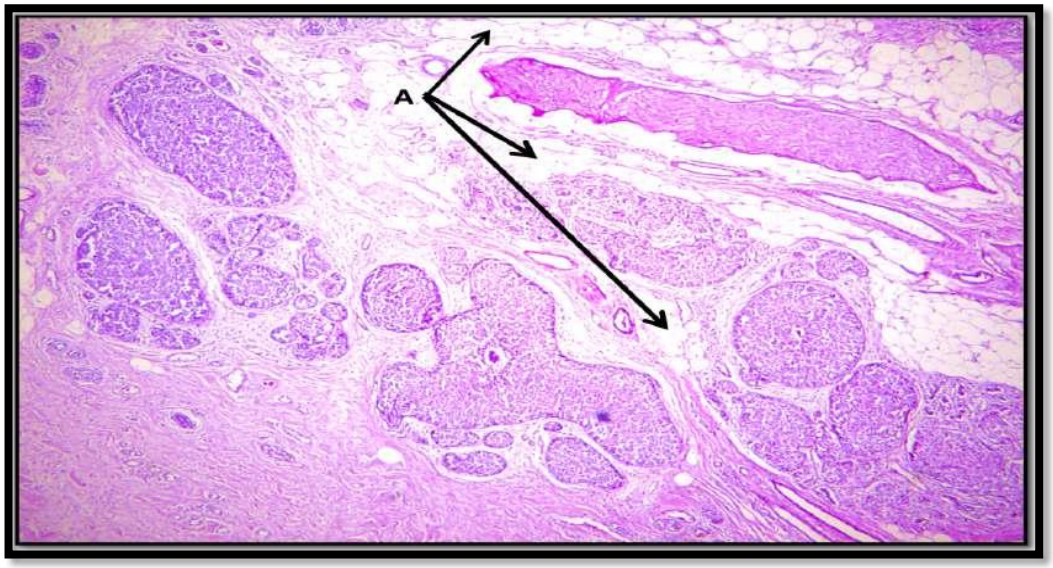



**Figure (6): Tissue section of breast cancer (30-39y) shows : A- abnormal cellular proliferation,B- blood congestion, C- tissue fibers D- thick secretion, H&E (10X).**

The tissue field showed prominent adipose tissue degeneration, most of adipocyte rupturing which lead to irregular spaces in location of adipose degeneration. Figure (7).




**Figure(7): Tissue section of breast cancer (40-49y) shows A- adipose tissue degeneration H&E(4X).**

The majority of secretory gland clusters within the breast tissue exhibited a decreased quantity when compared to the control groups. Additionally, most of these gland clusters lost their typical shape. Distinguishing between individual gland clusters within a single lobule became challenging, as all of them appeared as a single mass of cells. The cells that remained intact displayed irregular nuclei with a dark hue. Furthermore, a significant number of gland clusters lost their central cavities due to abnormal cellular growth. Figure (8).

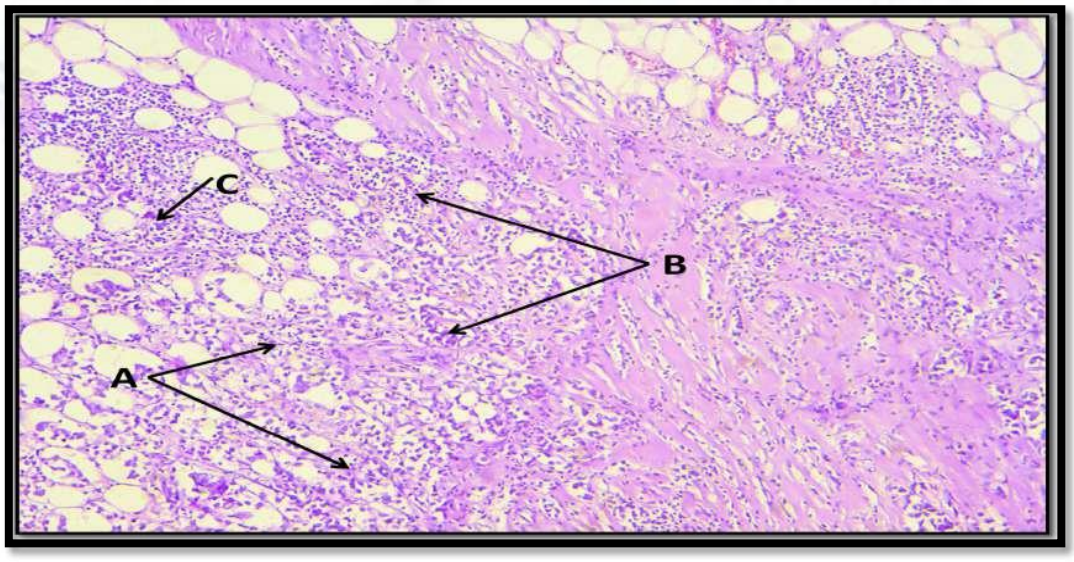



**Figure (8) :Tissue section of breast cancer (50-60y) shows A- abnormal cellular proliferation, B- secretory acini, C- irregular nuclei H&E (10X).**

**IMMUNOHISTOCHEMICAL**

**Ki67 antibody kit :**

In individuals aged 30-39 with breast infections, the immunohistochemistry analysis using the Ki67 kit revealed abnormal cell proliferation in the infected breast tissue, as depicted in Figure (10). The findings indicated positive staining in breast cells when using the Ki67 kit, signifying a significant variance in cell proliferation between the breast cancer group and the control group. In the case of breast tissue from individuals aged 40-49, a notably higher cell proliferation score was observed compared to both the previous infected and control groups, as shown in Figure( 9,11). Conversely, for individuals over the age of 50, there was a marked decrease in cell proliferation, which was considerably lower than that observed in the 30-39 age group, as illustrated in Figure ( 12 ,13).

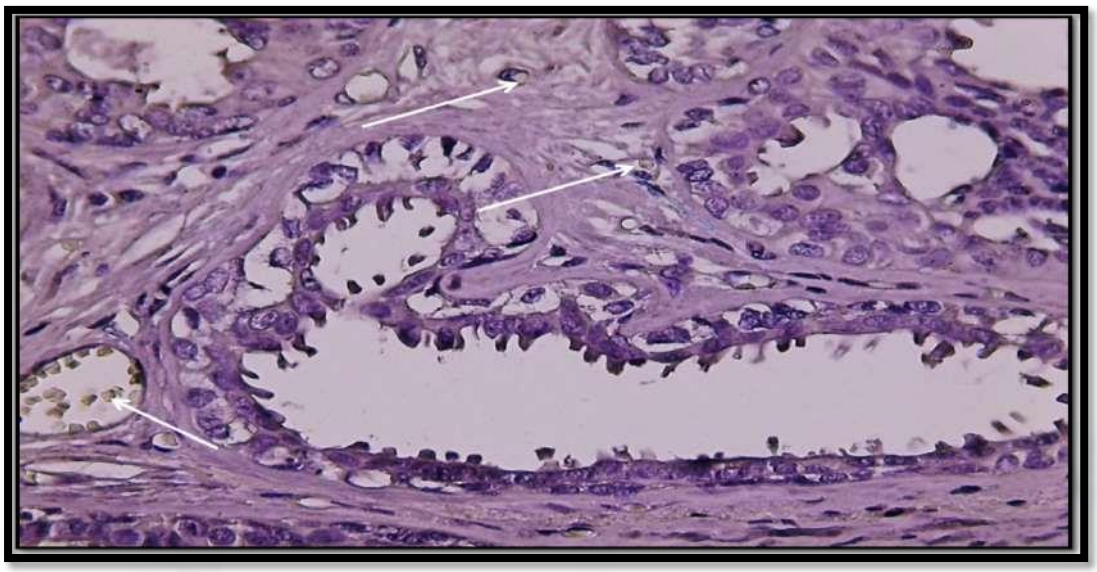


**Figure (9):Show the expression of ki67 in healthy group by using IHC technique.The brown color refers to positive stain (40X).**

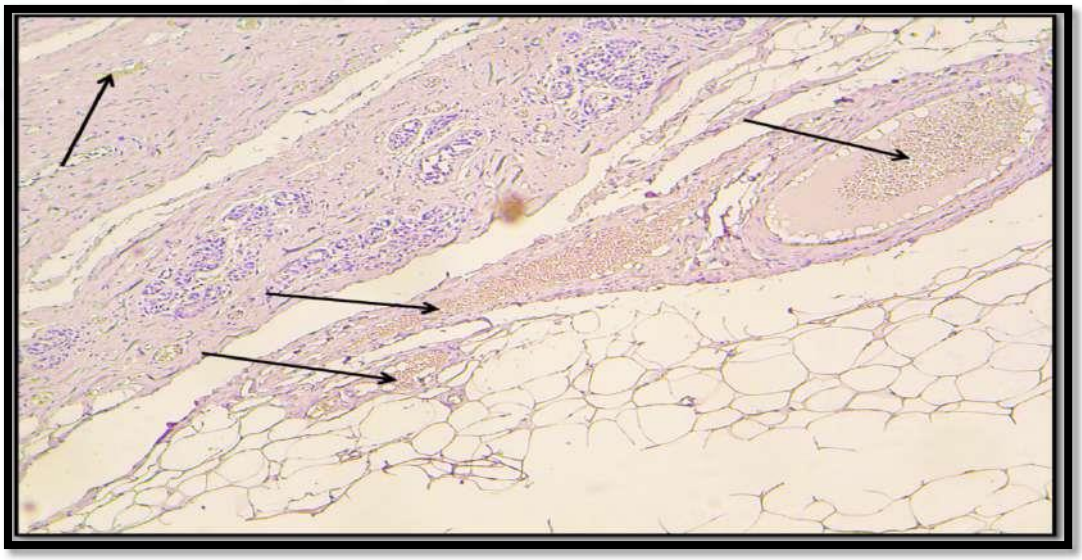


**Figure (10) : Show the expression of ki67 in patients group for age (30-39) by using IHC technique. The brown color refers to positive stain (4X).**

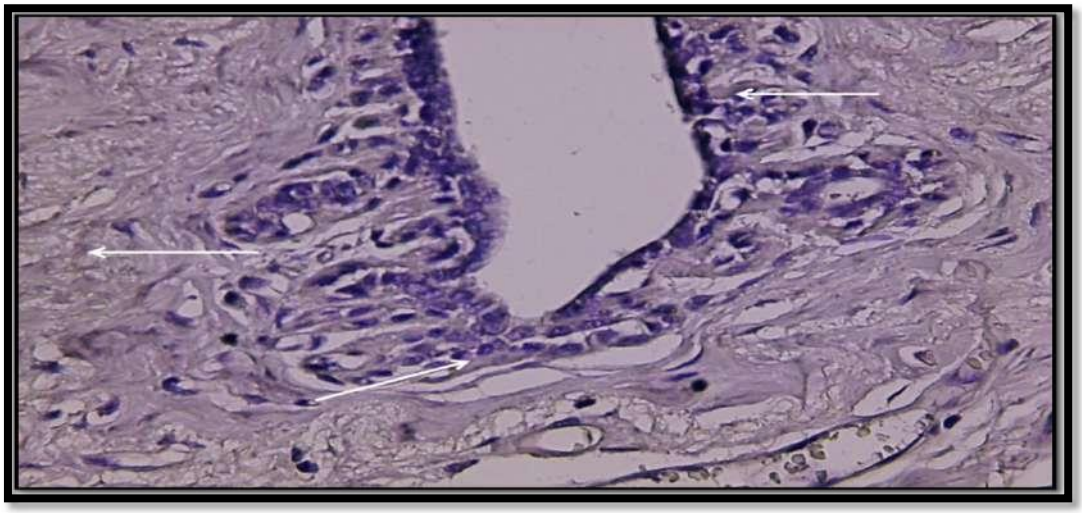


**Figure (11) : Show the expression of ki67 in patients group for age (40-49) by using IHC technique. The brown color refers to positive stain (40X).**

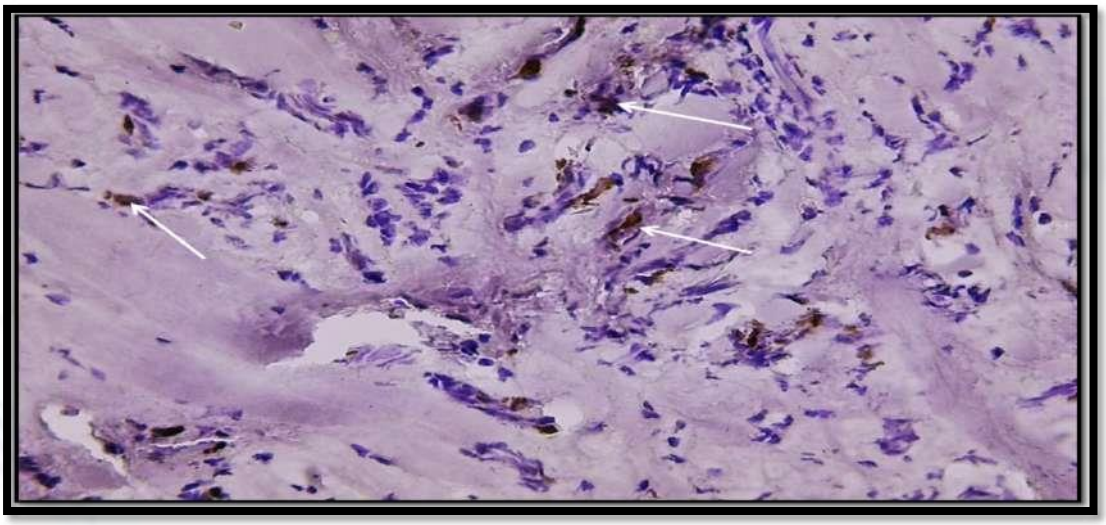


**Figure (12) : Show the expression of ki67 in patients group for age (50-60) by using IHC technique. The brown color refers to positive stain (40X).**

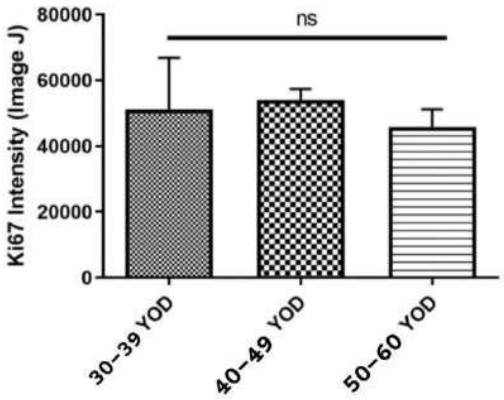


**Figure (13): Immunohistochemical intensity of (Ki67): 30-39 YOD group: patients' age ranged from 30-39 years old. 40-49 YOD group: patients' age ranged from 40-49 years old. 50-60 YOD group: patients' age ranged from 50-60 years old. Immunohistochemical intensity was measured by using ImageJ software (Image Processing and Analysis in Java). Ordinary one-way ANOVA and Newman-Keuls post hoc test. ns non-significant (P>0.05). N=20**

**Progesterone receptor kit**

The results from immunohistochemistry performed on breast tissue infected with the progesterone receptor kit revealed abnormal scores for positive staining cells. Notably, there was a significant difference in cell proliferation observed in infected breast tissue from individuals aged 30-39 when compared to the control group as shown in (Figure 15). Moreover, within the 40-49 age group, the tissue sections displayed a notably high level of cellular proliferation scores, surpassing both the earlier infected and control groups .Figure (16). Conversely, in the 50-60 age group, the tissue sections of breast cancer exhibited a considerably elevated proliferation score, though it was lower than that of the infected breast tissue in the 40-49 age group and higher than both the 30-39 age group and the control groups .Figure (14,17,18).

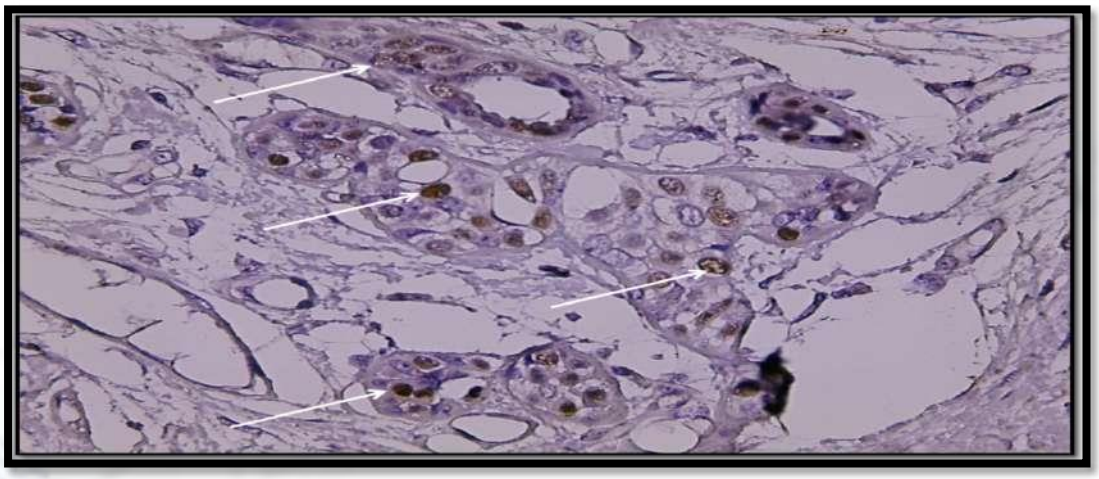


**Figure (14) : Show the expression of progesterone in healthy group by using IHC technique. The brown color refers to positive stain (40X).**

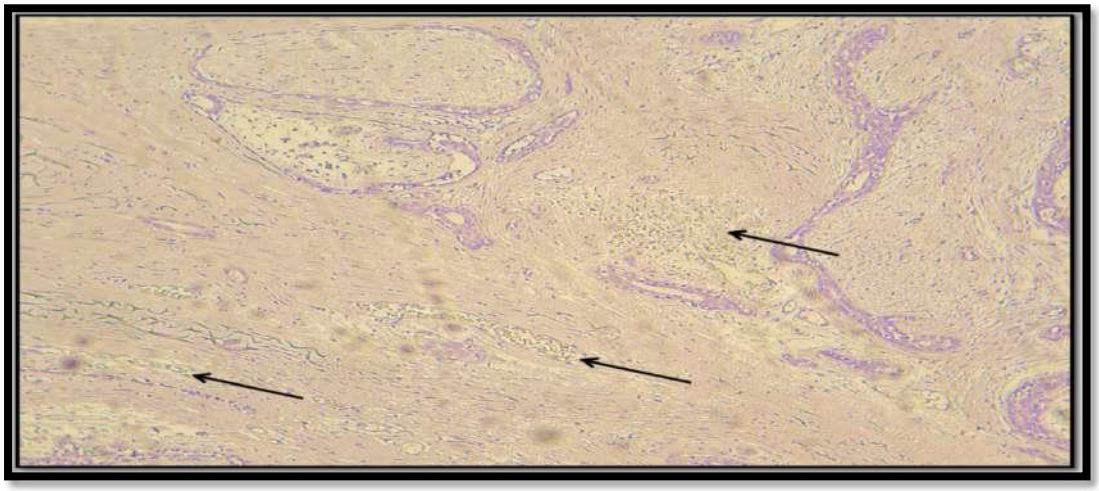


**Figure (15) : Show the expression of progesterone in patients group for age (30-39) by using IHC technique. The brown color refers to positive stain (40X).**

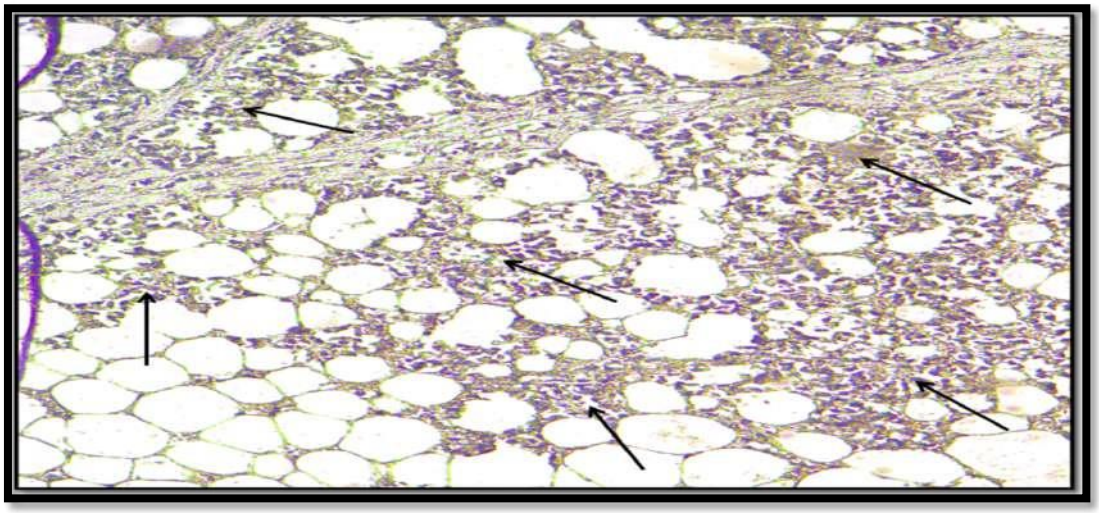


**Figure (16) : Show the expression of progesterone in patients group for age (40-49) by using IHC technique. The brown color refers to positive stain (4X).**

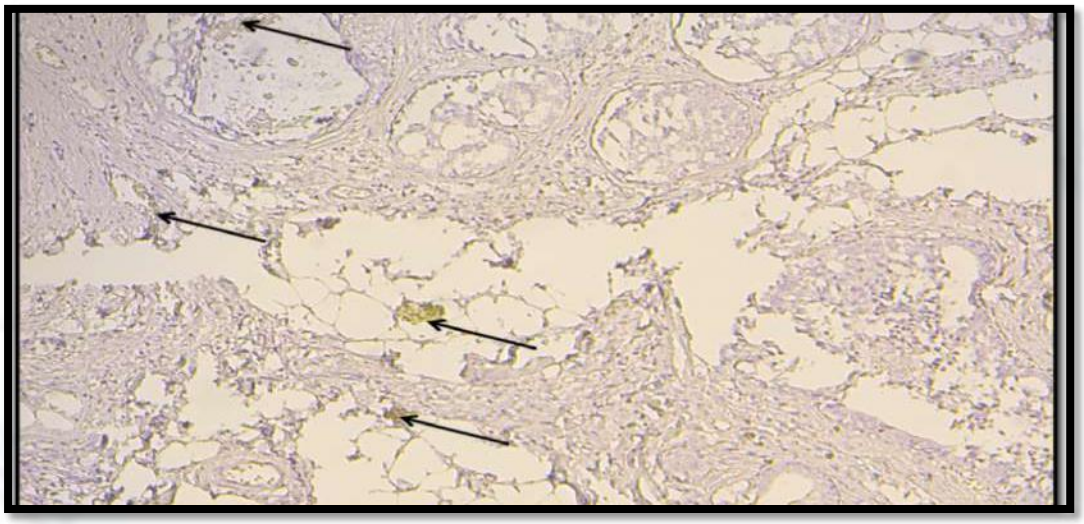


**Figure (17) : Show the expression of progesterone in patients group for age (50-60) by using IHC technique. The brown color refers to positive stain (4X).**

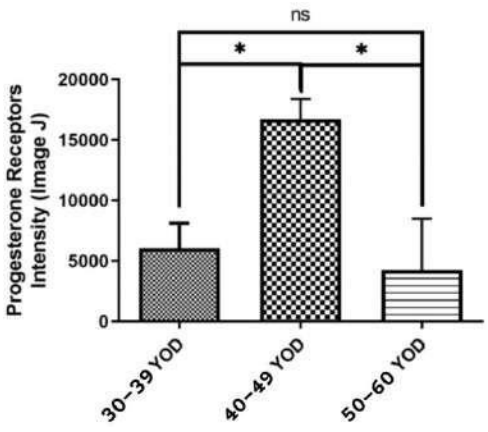



**Figure (18): Immunohistochemical intensity of (Progesterone Receptors): 30-39 YOD group: patients' age ranged from 30-39 years old. 40-49 YOD group: patients' age ranged from 40-49 years old. 50-60 YOD group: patients' age ranged from 50-60 years old. Immunohistochemical intensity was measured by using ImageJ software (Image Processing and Analysis in Java). Ordinary one-way ANOVA and Newman-Keuls post hoc test. ns non-significant (P>0.05). N=20.**

**Discussion:**

An extremely aggressive form of cancer is often linked to the onset of breast cancer at a younger age. Our research indicated that the majority of cases fell within the 30 to 49 age group, accounting for 47.5% of all patients. This finding aligns with outcomes observed in other studies that have identified Iraq as a nation with a particularly high risk of breast cancer. The heightened incidence of breast cancer in southern regions like Basra and Thi-Qar may be linked to the complex sociopolitical circumstances in Iraq, including factors such as chemical warfare and bombings[10]. Moreover,our study found that about 67.5% of the women affected by breast cancer resided in urban areas, while approximately 32.5% lived in rural regions. These findings are consistent with the results of other research studies [11], several factors may contribute to these outcomes, the higher population density in urban areas, coupled with ongoing emissions of gases and smoke from industrial facilities and factories concentrated in these regions, as well as the frequent use of fossil-fuel-powered vehicles and the limited presence of agricultural activities, contrast with the relatively cleaner and more natural environment of rural areas. In our research, we observed a smaller proportion of patients (22.5%) who had a positive family history compared to a larger percentage (77.30%) with a negative family history,the findings were in agreement with an earlier study, which suggested that the increased risks linked to a positive family history could be attributed to a variety of genetic factors. These genetic elements have the potential to influence the emergence of both estrogen receptor positive (ER-positive) and estrogen receptor negative (ER-negative) tumors [12]. The results of the study agree with numerous studies that found ,using oral contraceptives currently was linked to an increased risk of invasive breast cancer, irrespective of the specific disease subtype. However, the risk in those who had stopped using them was similar to those who had never used them, five years after discontinuation. When considering different progestin types, associations were found for specific formulations containing levonorgestrel and norgestrel. Research on newer progestin types like desogestrel, norgestimate, and drospirenone was limited due to the sample size, emphasizing the need for further studies on recently introduced progestins[13].

These results align with earlier research indicating that infections prompt the immune system to release signaling molecules, which can result in unusual cellular growth within breast tissue. This mechanism can cause alterations in tissue architecture, including the development of fibrotic changes. Inflammatory responses can also lead to the production of thick secretions, injury to blood vessels, and an increase in their permeability, ultimately resulting in the release of blood into adjacent tissues [14,15].

These findings align with the research that stated that fat necrosis occurs when adipose tissue is injured, resulting in inflammation and the release of fat cells. Additionally, breast fat necrosis can be induced by radiation therapy [16]. These findings align with a previous study that demonstrated similar alterations, including the absence of acinar differentiation, irregular nuclei, and abnormal cell proliferation, which are frequently observed characteristics in advanced breast carcinoma. The presence of isolated cellular masses may be indicative of the advancement of invasive growth [17]. This result similar to study which showed the correlation between age and the levels of positive expression of Ki67, a nuclear protein utilized for quantifying proliferating cells, shows a notable link. Ki67 expression elevation corresponds to increased cell growth. Research indicates that breast carcinomas with Ki-67 positive cells advance to higher histological grades, suggesting a role in tumor progression. The heightened expression of Ki67 is closely tied to unfavorable clinicopathological factors like advanced age, high-grade tumors, and lymph node metastasis, further implying its association with poor prognosis in breast cancer cases [18]. This result similar to study that showed ,substantial evidence suggests that positive expression of progesterone receptors (PR) is more prominent in younger women, signifying a more aggressive form of breast carcinoma within this age group. Furthermore, heightened exposure to progesterone is associated with an elevated risk of breast cancer, contributing to the development of both hormone receptor-negative and hormone receptor-positive tumors. Postmenopausal women with elevated levels

of circulating progesterone face a 16% increased risk of breast cancer. In most target tissues, PR functions as adirect estrogen-inducing gene, potentially collaborating with or counteracting estrogen receptors (ER) to impact downstream biological processes. In promoter interference assays, PR primarily suppresses ER transcription activity through PR-a[19].

**Conclusions:**

In our research results and in line with epidemiological observational studies, multiple authors have corroborated a minor increase in the risk of breast cancer in women who use contraceptives when compared to those who abstain from using themwe observed significant histological and morphological changes in the breast tissue compared to the control breast. These included abnormal cell proliferation, enlargement of breast structures, the presence of abnormal cell clusters, congested blood vessels, elongated areas with intermittent bleeding, necrotic lesions, and various other noticeable changes, all of which indicate the presence of breast cancer. The findings from immunohistochemistry revealed a substantial variation in cell proliferation between the healthy and infected groups. Specifically, the immunohistochemical results, utilizing ki67 and progesterone antibody kits, demonstrated elevated cellular proliferation in females diagnosed with breast cancer in comparison to the control group.

**Reference:**


1. Siegel, R. L., Miller, K. D., & Jemal, A. (2019). Cancer statistics, 2019. CA: a cancer journal for clinicians, 69(1), 7-34
2. Houssein, E. H., Emam, M. M., Ali, A. A., & Suganthan, P. N. (2021). Deep and machine learning techniques for medical imaging-based breast cancer: A comprehensive review. Expert Systems with Applications, 167, 114161.
3. American Cancer Society (2019 b). Breast Cancer Facts & Figures 2019-2020. Atlanta:American Cancer Society, Inc:38-71.
4. Chattopadhyay, S., Dey, A., Singh, P. K., & Sarkar, R. (2022). DRDA-Net: Dense residual dual-shuffle attention network for breast cancer classification using histopathological images. Computers in biology and medicine, 145, 105437.
5. American Cancer Society (2014): Breast cancer. American Cancer Society(2015): Breast Cancer Facts & Figures 2015- 2016.Atlanta:American cancer society, Inc. 38pp.
6. Łukasiewicz, S., Czeczelewski, M., Forma, A., Baj, J., Sitarz, R., & Stanisławek, A. (2021). Breast cancer—epidemiology, risk factors, classification, prognostic markers, and current treatment strategies—an updated review. Cancers, 13(17), 4287.
7. Iversen, L., Sivasubramaniam, S., Lee, A. J., Fielding, S., & Hannaford, P. C. (2017). Lifetime cancer risk and combined oral contraceptives: the Royal College of General Practitioners' Oral Contraception Study. American journal of obstetrics and gynecology, 216(6), 580-e1.
8. d'Amati, A., Mariano, M., Addante, F., Giliberti, G., Tomasicchio, G., & Mastropasqua, M. G. (2022). When Histological Tumor Type Diagnosed on Core Biopsy Changes Its Face after Surgery: Report of a Deceptive Case of Breast Carcinoma. Reports, 5(4), 38.
9. Kabiraj, A., Gupta, J., Khaitan, T., & Bhattacharya, P. T. (2015). Principle and techniques of immunohistochemistry—a review. Int J Biol Med Res, 6(3), 5204-5210.
10. Alwan, N. A. S. (2010). Breast cancer: demographic characteristics and clinico-pathological presentation of patients in Iraq. EMHJ-Eastern Mediterranean Health Journal, 16 (11), 1159-1164, 2010..
11. Aynalem, M., Adem, N., Wendesson, F., Misganaw, B., Mintesnot, S., Godo, N., ... & Shiferaw, E. (2022). Hematological abnormalities before and after initiation of cancer treatment among breast cancer patients attending at the University of Gondar comprehensive specialized hospital cancer treatment center. Plos one, 17(8), e0271895

12. Khalaf, H., Mohammed, A., Shukur, S., Alhalabi, N., Almothafar, B., Hassan, M., & Abu, A. (2022). Breast cancer: age incidence, hormone receptor status and family history in Najaf, Iraq. Journal of Medicine and Life, 15(10), 1318.

13. (13) Burchardt, N. A., Eliassen, A. H., Shafrir, A. L., Rosner, B., Tamimi, R. M., Kaaks, R., ... & Fortner, R. T. (2022). Oral contraceptive use by formulation and breast cancer risk by subtype in the Nurses' Health Study II: a prospective cohort study. American Journal of Obstetrics and Gynecology, 226(6), 821-e1

14. Farhood, B., Hoseini-Ghahfarokhi, M., Motevaseli, E., Mirtavoos-Mahyari, H., Musa, A. E., & Najafi, M. (2020). TGF-β in radiotherapy: Mechanisms of tumor resistance and normal tissues injury. Pharmacological research, 155, 104745

15. Munn, L. L. (2017). Cancer and inflammation. Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 9(2), e1370..

16. Vasei, N., Shishegar, A., Ghalkhani, F., & Darvishi, M. (2019). Fat necrosis in the Breast: A systematic review of clinical. Lipids in health and disease, 18(1), 1-9

17. Ajkunic, A., Skenderi, F., Shaker, N., Akhtar, S., Lamovec, J., Gatalica, Z., & Vranic, S. (2022). Acinic cell carcinoma of the breast: A comprehensive review. The Breast.

18. Zhao, X., Yang, X., Fu, L., & Yu, K. (2021). Associations of estrogen receptor, progesterone receptor, human epidemic growth factor receptor-2 and Ki-67 with ultrasound signs and prognosis of breast cancer patients. Cancer Management and Research, 4579-4586

19. (19) Li, Z., Wei, H., Li, S., Wu, P., & Mao, X. (2022). The role of progesterone receptors in breast cancer. Drug Design, Development and Therapy, 305-314.